

Measurement of Hematological, Clinical Chemistry, and Infection Parameters from Hirudinized Blood Collected in Universal Blood Sampling Tubes

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ABSTRACT

Hirudin, the anticoagulatory polypeptide of the leech *Hirudo medicinalis*, strongly inhibits thrombus formation by specifically interacting with thrombin. For diagnostic purposes, hirudin should be superior to other anticlotting compounds because it only minimally alters the mineral, protein, and cellular blood constituents. To test this hypothesis, hirudinized and routinely processed venous blood from 80 healthy volunteers and patients was subjected to a variety of automated blood tests. A strong correlation was found between the results of automated complete blood counts obtained from K₂-ethylenediaminetetraacetic acid (EDTA) anticoagulated and hirudinized blood (1000 antithrombin units [ATU] hirudin/ml). In addition, clinical chemistry and serological infection parameters (aspartate aminotransferase [ASAT], lactate dehydrogenase [LDH], sodium, and so on, and antibodies against hepatitis B and C and human immunodeficiency virus [HIV]1/2, respectively) correlated well when measured in serum as compared with hirudinized plasma. Contrary to single clotting factors, global coagulation parameters (activated partial thromboplastin time [aPTT], prothrombin time [PT]) could not be measured in hirudinized blood. Recombinant hirudin neither interfered with immunophenotyping of mononuclear cells using FACScan analysis, nor did it alter the detection of Wilms' tumor gene expression by RT-PCR technology even at high doses (5000 ATU hirudin). Thus, a hirudin-containing blood sampling tube can be designed as a universal blood sampling tube (UBT) for testing the majority of diagnostic blood parameters.

KEYWORDS: Recombinant hirudin, universal blood sampling tube (UBT), automated blood count, clinical chemistry parameters, immunophenotyping, RT-PCR

Objectives: Upon completion of this article, the reader should be able to (1) list some of the clinical laboratory parameters that can be reliably tested in hirudin-containing blood sampling tubes and (2) recognize some of the advantages of using hirudin-containing sampling tubes.

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Hirudo medicinalis, the European leech, has long been recognized for its blood-diluting properties. The ant clotting properties in its salivary glands were first recognized in 1884.^{1,2} Twenty years thereafter, a crude anticoagulant compound was isolated from leeches, and later, in 1927,³ the antithrombotic properties of hirudin preparations and heparin were compared for the first time. In the late 1950s, Markwardt systematically purified leech anticoagulants and characterized their polypeptide structure.⁴ The establishment of efficient large-scale production processes for recombinant hirudin⁵⁻¹⁰ has led to its development as a pharmaceutical anticoagulant (e.g., Refludan[®], Revasc[®]) for indications such as deep venous thrombosis, heparin-induced thrombocytopenia, and coronary syndromes. The anticoagulatory action of natural and recombinant desulfato-hirudin was shown to be similar.^{11,12}

Natural hirudin, a single-chain, carbohydrate-free polypeptide, has a molecular weight of 7kD. It is composed of 65 amino acids and contains three intramolecular disulfide bridges and a sulfated tyrosine residue. Hirudin interacts specifically with thrombin without the involvement of cofactors. With its C terminal tail, hirudin irreversibly binds to the fibrinogen recognition site of thrombin,¹³ effectively blocking the transformation of fibrinogen to fibrin.^{14,15} In contrast to the heparin-antithrombin (AT) complex that exclusively inhibits alpha- and gamma-thrombin, hirudin also inhibits the enzymatic function of meizothrombin, a precursor of alpha-thrombin.¹⁶ Because of its highly specific interaction with thrombin, hirudin can be expected to have a relatively low interference with diagnostic test systems or other soluble blood components and may therefore be preferable to other anticoagulants.

We compared a variety of diagnostic data obtained from hirudinized and routinely processed blood of patients and healthy volunteers. In addition to an automated complete blood count, automated clinical chemistry, and serological infection parameters, immunophenotyping of mononuclear cells (MNCs) and Wilms' tumor gene (WT1) expression data (RT-PCR technology) were analyzed for differences attributable to the anticoagulant used.

MATERIALS AND METHODS

Patients

Venous blood was drawn from healthy volunteers (n = 35) and hospitalized patients (n = 45). The patients were treated for a variety of life-threatening diseases,

such as renal and liver failure, myocardial infarction, sepsis, and leukemia.

Universal Blood Sampling Tubes

Recombinant desulfato-hirudin was produced in *Hansenula polymorpha*. Lyophilized hirudin with a specific antithrombotic activity (ATU) of 19,000 per milligram was dissolved in distilled water to prepare a hirudin working solution containing 100 ATU/ μ L (method according to Griessbach et al¹⁷). A working solution of hirudin was added to plain glass tubes by pipette, resulting in a final concentration of 1000 ATU/mL after being filled with 4 mL freshly drawn blood. As controls, plastic Vacutainer[®] blood sampling tubes containing K₂-EDTA (4-mL tube; for blood cell counting and PCR tests), glucosidase inhibitor (3-mL tube; to assess serum glucose levels), clot activator, and separation gel (8.5-mL tube; serum tube for measuring clinical chemistry parameters and serological virological testings), or heparin (10-mL tube; for immunophenotyping) were used. All tubes were purchased from Becton Dickinson (Heidelberg, Germany). The prepared blood sampling tubes were filled with venous blood using a butterfly venipuncture system (Becton Dickinson), as described.¹⁸

Routine Blood Tests

All routine blood parameters were measured within a 6-hour time frame after venipuncture. The K₂-EDTA-tubes and UBTs were moved gently to prevent thrombocyte microaggregation. Blood tubes were transported and stored at room temperature. The UBTs were first analyzed for the complete blood count (including differential) using the automated blood counter STK (Beckman Coulter, Krefeld, Germany). Subsequently, the UBTs were centrifuged (2000 rpm). Hirudinized plasma was taken to automatically measure a variety of clinical chemistry parameters (albumin, alkaline phosphatase, amylase, total bilirubin, calcium, chloride, cholesterol, creatine kinase, creatinine, gamma glutamyltransferase [GGT], glucose, ASAT, alanine aminotransferase [ALAT], hydroxybutyrate dehydrogenase [HBDH], LDH, lipase, p-amylase, phosphate, potassium, sodium, total protein, triglyceride, urea, and uric acid) using the Roche/Hitachi 917 System[®] (Roche Diagnostics, Mannheim, Germany). This analyzer is a flexible system for the measurement of both routine and special clinical chemistry parameters in blood samples. It is based on ion-selective electrodes (Na⁺, K⁺, Li⁺) and absorbance measurements in reaction solutions consist-

ing of reagent-mixed serum (or in our case hirudinized plasma) in a plastic cuvette. A minimum sample volume between 2 and 35 μL was required for each test parameter. This analyzer was operated according to the manufacturer's instructions using routine test kits from Roche Diagnostics AG. In addition, the special clinical chemistry parameters free hemoglobin (measured by photometry in a Lange photometer), thyroxine-stimulating hormone (by electrochemiluminescence-detection technology, Elecsys System[®] 1010, Roche Diagnostics), and thyroglobulin (by radioimmunoassay, Perkin Elmer Wallace, Freiburg, Germany) were quantified from the blood of a small group of volunteers ($n = 6$). These parameters were selected as examples of different types of detection methods. No adaptations were made for measuring hirudinized blood.

Immunophenotyping

Bone marrow or blood samples were anticoagulated with desulfato-hirudin at a concentration of 1000 ATU/mL or heparin (standard procedure) for 24 hours. MNCs of two patients with acute myelogenous leukemia (AML) (bone marrow), one patient with chronic myelocytic leukemia (CML) in the chronic phase (blood), one patient with chronic lymphocytic leukemia (CLL) (blood), and one patient with sepsis (reactive bone marrow) were isolated by Ficoll-gradient centrifugation. MNCs were immunophenotyped using FAC-Scan analysis (Becton & Dickinson, Software Cellquest) with monoclonal antibodies from Beckman Coulter Immunotech (Heidelberg, Germany) (SJ1D1 [CD13], D3HL60-251 [CD33], 80H5 [CD15], 88H7 [CD65], B8.12.2 [HLA-DR], RMO52 [CD14], 13B8.2 [CD4], B9.11 [CD8], 95C3 [CD117]) and from Becton Dickinson (581 [CD34], RPA-T4 [CD4], M-T701 [CD7]). Immunophenotyping was repeated twice.

Enzyme-Linked Immunoassays

Using the third-generation immunoassay system AxSYM[®] (Abbott Systems, Abbott Park, IL), serological tests were performed with serum and hirudinized plasma from eight patients to detect antibodies against cytomegalovirus (CMV), hepatitis B and C, and HIV1/2. The Abbott test kits used were CMV-immunoglobulin G (IgG), AUSAB, HAVAB, hepatitis C virus (HCV), and HIV AySym1/2 gO.

PCR Technology

Testing for hepatitis C viral genomes was performed using COBAS-Amplicor HCV V.2.0[®] from Roche Diagnostics. The commercially available and approved HCV-RNA preparation #75/98 served as positive reference. An internal standard was coamplified in each sample (positive PCR control). The test was performed

according to manufacturer's instructions. Hirudinized plasma at a concentration of 1000 ATU hirudin/mL was used.

To detect WT1, total RNA was prepared from 10^6 EoL1 leukemic blasts, known for overexpressing WT1. To evaluate whether hirudin interferes with the RT-PCR technique, 500, 1000, 2500, and 5000 ATU hirudin were added directly to 10^6 pelleted EoL1 blasts. The cell suspension was shaken vigorously. After 1 hour of incubation, total RNA was isolated according to the RNazol B-protocol. Reverse transcription was performed using M-MLV Reverse Transcriptase (Life Technologies, Karlsruhe, Germany). RNA integrity of each sample was determined by amplifying c-ABL cDNA.¹⁸ According to this RT-PCR-protocol, a 487-bp DNA fragment including the WT1 zinc finger region (365 bp) was detected after two amplification rounds using two different primer sets.¹⁹ The protocol detects WT1 mRNA transcripts in 50 HL60 blasts interspersed among 10^6 normal pB MNCs.²⁰ This assay was performed in duplicates.

Statistical Methods

Correlation between the measurements obtained from routinely processed and hirudinized blood from each individual blood donor was calculated according to Spearman²¹ using the computer program SPSS (Version 6.1.1; SPSS Inc., Chicago, IL).

RESULTS

Comparative Analysis of Complete Blood Counts

Blood from healthy individuals or patients ($n = 80$) was drawn into one UBT and one standard K_2 -EDTA tube at the same time via the same venipuncture set. From both tubes automated complete blood counts were prepared. The absolute results of automated complete blood counts measured from hirudinized and K_2 -EDTA anticoagulated blood were only slightly different (data not shown). For complete blood counts, significant correlations and intraclass correlations (according to Spearman's test) were found between the measurements of each parameter from hirudinized and K_2 -EDTA-anticoagulated blood. However, the significance level of correlation was substantially lower for monocytes and basophils (both $p = 0.5$) as compared with the other blood cells (Table 1). Occasionally, some monocytes were mistaken for basophils during automated blood counting, sometimes resulting in paradoxically high basophil counts. This occurred somewhat more frequently in hirudinized anticoagulated blood than it did in K_2 -EDTA anticoagulated blood (7 and 2 of 73 samples, respectively). This problem was resolved by taking repeated measurements from the same tube.

Table 1 Correlation of Complete Automated Blood Counts Obtained from UBTs and Standard K₂-EDTA Tubes

	Number of Samples (n)	Correlation (R; Spearman)	Intraclass Correlation (ICR; Spearman)
Erythrocytes/pL	80	0.9904***	0.9902***
Leukocytes/ μ L	80	0.9527***	0.9464***
Thrombocytes/nL	80	0.9158***	0.9085***
Hemoglobin g/dL	80	0.9887***	0.9886***
Neutrophils (%)	78	0.8911***	0.8739***
Lymphocytes (%)	78	0.9654***	0.9652***
Monocytes (%)	78	0.7468***	0.508*
Eosinophils (%)	78	0.8777***	0.8775***
Basophils (%)	77	0.3472*	0.2408*

***Significant at level $p < 0.001$; **significant at level $p < 0.01$; *significant at level $p < 0.05$.

All measurements from blood of healthy volunteers were within normal range, irrespective of the anticoagulant used. In addition, the measurements from patients' hirudinized blood indicated the same diagnostic conclusion as K₂-EDTA-anticoagulated blood for each of the diseases (e.g., thrombocytopenia, leukocytosis; data not shown).

Comparative Analysis of Routine Clinical Chemistry Parameters

Blood of healthy individuals and hospitalized patients ($n = 64$ to 74) was collected in a UBT and a regular serum tube, centrifuged, and subjected to an automated analyzer. The overall results of 24 clinical chemistry pa-

Table 2 Correlation of Clinical Chemistry Parameters Measured in Serum and Hirudinized Plasma

Test Parameter	Number of Samples (n)	Correlation (R; Spearman)	Intraclass Correlation (ICR; Spearman)
GOT	74	0.9904***	0.9986***
GPT	65	0.9973***	0.8004***
AP	64	0.9884***	0.9874***
Gamma GT	65	0.9726***	0.9717***
HBDH	65	0.9829***	0.9825***
Bilirubin (total)	74	0.9178***	0.914***
CK	65	0.9969***	0.9956***
Lipase	65	0.9808***	0.9801***
LDH	65	0.9751***	0.973***
Amylase	65	0.9958***	0.9952***
Creatinin	64	0.9768***	0.9766***
Urea	64	0.9963***	0.9937***
Uric acid	65	0.9964***	0.9956***
Phosphate	64	0.9627***	0.9278***
Glucose	64	0.9223***	0.9169***
Cholesterol	65	0.9936***	0.9895***
Triglyceride	65	0.9412***	0.9223***
Calcium	72	0.8246***	0.8239***
Total protein	73	0.9113***	0.8841***
Albumine	65	0.9855***	0.9849***
Pancreatic amylase	65	0.9952***	0.9949***
Potassium	64	0.8904***	0.7536***
Sodium	73	0.8857***	0.8394***
Chloride	65	0.8782***	0.8622***

Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

rameters were only slightly different when measured from hirudinized plasma instead of serum (data not shown). A strong correlation between the respective measurements from serum and hirudinized plasma was found in Spearman's correlation and intraclass correlation tests (significance level $p < 0.001$) (Table 2). The measurements of potassium in hirudinized plasma were consistently lower, whereas total protein was always found at higher concentrations in hirudinized plasma as compared with serum. Again, all clinical chemistry parameters of healthy volunteers were within normal range and the pathological parameters of the patients had the same indicative power for their particular disease, irrespective of whether serum or hirudinized plasma was analyzed (e.g., increased urea levels—indicating renal failure; data not shown).

Special Clinical Chemistry Parameters

In healthy volunteers ($n = 8$), free hemoglobin in hirudinized plasma was repeatedly found at substantially lower concentrations than it was in serum. The difference between both measurements of free hemoglobin was between 43 and 98% (Table 3). All measurements were still within normal range. For TSH and thyroglobulin, comparable measurements were obtained in healthy volunteers ($n = 9$) (Table 3).

Infection Serological Parameters

From serum and hirudinized plasma of a small group of healthy volunteers ($n = 6$), automated detection of antibodies against CMV, hepatitis B ($n = 9$), hepatitis C, and HIV1/2 as well as detection of hepatitis B surface

Table 3 Special Clinical Chemistry and Infection Serological Parameters

Patients/ Material	Clinical Chemistry Parameters			Infection Serological Parameters (no.)					PCR
	Free Hemoglobin (mg/L)	TSH (mU/mL)	Thyreo- globulin- (μ g/L)	CMV IgG-AB	Anti- Hbs-AB (IU/L)	HBs- AG	HCV- AB	HIV1/2- AB	HCV- genomic (ctl/hcv)
1/ Serum	78.62	2.02	5.6	—	>1000	—	—	—	+/-
H-plasma	24.95	2.03	4.9	—	>1000	—	—	—	+/-
Deviation (%)	-68.3	+0.5	-12.5	0	0	0	0	0	0
2/ Serum	54.52	1.03	3.8	+	—	—	—	—	+/-
H-plasma	31.08	1.14	4.4	+	—	—	—	—	+/-
Deviation (%)	-43	+8.7	-16	0	0	0	0	0	0
3/ Serum	38.30	2.22	13	+	—	—	—	—	+/-
H-plasma	21.84	2.25	7.4	+	—	—	—	—	+/-
Deviation (%)	-43	+1.4	-43	0	0	0	0	0	0
4/ Serum	77.87	2.18	7	—	>1000	—	—	—	+/-
H-plasma	23.94	2.47	8.3	—	>1000	—	—	—	+/-
Deviation (%)	-69.3	+13.3	+18.6	0	0	0	0	0	0
5/ Serum	131.50	0.82	5.1	—	—	—	—	—	+/-
H-plasma	2.18	0.85	5.8	—	—	—	—	—	+/-
Deviation (%)	-98	+3.7	+13.7	0	0	0	0	0	0
6/ Serum	130.8	1.72	11	+	—	—	—	—	+/-
H-plasma	32.42	1.78	11	+	—	—	—	—	+/-
Deviation (%)	-75	+3.5	0	0	0	0	0	0	0
7/ Serum	133.1	1.88	2.7	nd	6040	nd	nd	nd	nd
H-plasma	27.3	1.84	2.6	nd	1376	nd	nd	nd	nd
Deviation in%	-79	-2.2	+3.7		-77				
8/ Serum	129.1	1.26	11	nd	527	nd	nd	nd	nd
H-plasma	65.18	1.34	12	nd	339	nd	nd	nd	nd
Deviation in%	-50	+6.3	+9.1		-36				
9/ Serum	nd	1.93	4.6	nd	1595	nd	nd	nd	nd
H-plasma	nd	1.89	4.9	nd	1956	nd	nd	nd	nd
Deviation in%		-2	+6.5		+23				

#AB = antibodies; AG = antigen; ctl = internal control, coamplified in every sample; hcv = hepatitis C viral genome; + = detectable; - = nondetectable; nd = not done.

antigen was technically possible and gave similar results (Table 3).

Coagulation Parameters

Hirudinized blood could not be used for measuring the global coagulation parameters, aPTT, and PT (synonymous Quick's test). In both test systems, hirudinized blood did not clot even after adding an excess of tissue factor plus calcium (PT) and a PTT reagent (cephaloplastin or reagents containing platelet factor 3), respectively. However, single clotting factors such as fibrinogen and AT III could be measured from hirudinized blood (data not shown).

Immunophenotyping

Immunophenotyping was performed using FACScan analysis with a panel of different antibodies on MNC preparations obtained from patients with AML ($n = 2$), CML in chronic phase ($n = 1$), and CLL ($n = 1$) and from a patient with reactive bone marrow due to bacterial sepsis. After incubation of bone marrow or blood for 24 hours with heparin or hirudin, MNCs were isolated by Ficoll centrifugation and subjected to the analysis. Comparable percentages of antigen expressing MNCs were obtained, irrespective of pretreatment with heparin or hirudin (Table 4). In addition, CD4 and CD8 expressing lymphocytes could be easily counted in hirudinized blood using FACScan analysis. In five healthy volunteers, almost the same counts of CD4 and CD8 expressing T lymphocytes were obtained from hirudinized blood as from K_2 -EDTA anticoagulated blood (data not shown).

PCR Technology

To clarify whether hirudin interferes with the enzymatic reactions involved in the RT-PCR technology, different amounts of hirudin were added to EoL1 leukemic blasts. After incubation of 1 hour, RNA was extracted and transcribed to cDNA, and PCR was performed to detect WT1 gene expression. WT1 cDNA fragments were amplified in all preparations, even when up to 5000 ATUs of hirudin were added. No differences in the boldness of the WT1 amplification bands were observed for hirudin-containing preparations, as compared with native preparations (Fig. 1).

DISCUSSION

Since medieval times, hirudin has been known for its blood-diluting capacities, but its use in diagnostic procedures has not been evaluated so far. The major objective of this study was to find out whether hirudin would interfere with methods, test kits, and assays that are used routinely for measuring corpuscular and dissolved

blood constituents. Thus, we have performed a comparative study of the results of automated complete blood counts, routine clinical chemistry, and serological infection parameters obtained from hirudinized versus routinely processed blood. Furthermore, to detect interferences of hirudin with complex test systems, we immunophenotyped MNCs using FACScan analysis and tested leukemic blasts for WT1 expression by RT-PCR treated with high hirudin doses.

In a recent study, we showed that hirudin at a concentration of at least 300 ATU/mL is able to anticoagulate blood for at least 24 hours.¹⁸ Stocker et al, in a previous study, reported that 150, 300, and 1000 ATU/mL desulfato hirudin were necessary to sufficiently prevent blood clotting for 3.5, 6, and at least 24 hours, respectively.²² We used hirudin at a concentration of 1000 ATU/ml blood to better detect possible interactions between desulfato-hirudin and the procedures of automated blood counting as well as the kits and protocols for testing routine clinical chemistry parameters. Automated complete blood counts could be performed reproducibly from hirudinized blood. A significant correlation was found in the results of all parameters of the complete blood count obtained from UBTs and standard K_2 -EDTA tubes, even when blood of severely sick patients was analyzed.

All 24 routine clinical chemistry parameters could be reproducibly measured in hirudinized plasma and serum. For serum and hirudinized plasma, a significant correlation of the measurements of all clinical chemistry parameters was found, even in patients with severe pathologies such as terminal renal insufficiency, myocardial infarction, hypercalcemia and hyperglycemia. Some clinical chemistry parameters such as potassium were consistently measured at lower concentrations in hirudinized plasma than they were in serum. This phenomenon might be attributable in part to the volume difference between serum and plasma, approximately 4% in favor of plasma, resulting in a higher concentration of soluble compounds in serum. In addition, centrifugation of clotted blood to separate serum is known to lead to the destruction of at least some erythrocytes, resulting in an efflux of intracellular potassium into the serum. This is in concordance with our observation that free hemoglobin is detectable at much lower concentrations in hirudinized plasma as compared with serum. On the other hand, total protein is consistently found at higher concentrations in plasma as compared with serum because of the removal of clotting factors from serum. Although hirudinized plasma could not be used for global coagulation tests such as aPTT and PT, measurements of single clotting factors such as fibrinogen or AT III was possible. Serological infection parameters could be measured equally well from hirudinized plasma and serum. In addition, hirudin was not found to interfere with RT-PCR protocols even when high hirudin doses were used.

Table 4 Detection of Clusters of Differentiation Antigens (CD) on Heparinized and Hirudinized Mononuclear Cells (in %) from Patients with AML, CML, CLL, and Sepsis using FACScan Analysis

Patient/ Disease	Anti- coagulant	Myeloid Antigens*										B-Cell Antigens*					T-Cell Antigens					Progenitor Cell Antigens*		
		CD13	CD33	CD65	CD15	CD14	CD64	CD79a	CD19	CD20	CD23	slg	κ/λLC	CD7	CD5	CD4	CD34	CD10	HLA-DR					
1/	Heparin	96	52	40	8	11	5	6	nd	nd	nd	nd	nd	nd	nd	33	3	2	0					
AML-M2	Hirudin	90	50	37	5	9	3	3	nd	nd	nd	nd	nd	nd	nd	35	4	5	1					
2/	Heparin	73	45	5	3	2	5	nd	nd	nd	nd	nd	nd	nd	nd	3	55	2	67					
AML-M1	Hirudin	71	45	8	2	0	2	nd	nd	nd	nd	nd	nd	nd	nd	7	58	3	65					
3/	Heparin	51	36	85	92	13	33	0	nd	nd	nd	nd	nd	nd	nd	nd	1	6	0					
CML-CP	Hirudin	58	40	73	87	19	26	0	nd	nd	nd	nd	nd	nd	nd	nd	3	12	1					
4/	Heparin	nd	nd	nd	nd	nd	nd	85	78	76	80	+	0/80	nd	80	nd	nd	0	nd					
B-CLL	Hirudin	nd	nd	nd	nd	nd	nd	81	79	70	85	+	0/75	nd	82	nd	nd	0	nd					
5/	Heparin	29	88	86	74	13	85	nd	nd	nd	nd	nd	nd	8	nd	10	nd	1	15					
Sepsis	Hirudin	24	87	84	80	11	84	nd	nd	nd	nd	nd	nd	8	nd	12	nd	1	14					

*Expression of a selected cluster of differentiation antigens (CD) in percent of all analyzed mononuclear cells; antigen expression below 15% was considered as background fluorescence; nd = not done; AML = acute myeloid leukemia, CML = chronic myeloid leukemia, CP = chronic phase; B-CLL = chronic lymphocytic leukemia of B-lymphocytes; slg = surface immunoglobulin; κ/λLC = κ-light chain/λ-light chain of immunoglobulin.



Figure 1 Detection of WT1 mRNA transcripts in EoL1 blasts pretreated with hirudin. M = marker lane depicting DNA fragments of 1500 (top) to 500 bp (bottom) in 100-bp increments (Invitrogen, Karlsruhe, Germany). Lanes 1 to 5 show 487-bp WT1 amplicons of EoL1 blasts exposed to 0, 5000, 2500, 1000, and 500 ATUs of recombinant desulfato-hirudin, respectively. Lane 6 shows the water control.

Taken together, this feasibility study shows that hirudinized blood can be used to reproducibly measure automated blood counts including differentials and a great variety of clinical chemistry parameters, without necessitating major adaptations to the equipment and test kits currently used. Based on these findings, a UBT for diagnostic blood tests will possibly save costs by substantially reducing the number of blood sampling tubes and by simplifying handling and laboratory logistics. After all, it will minimize the diagnostic blood loss (medical vampirism) and associated inconveniences to our patients. Before general use of hirudinized blood for diagnostic purposes can be recommended, however, a pilot study with manufactured vacuumized UBTs should be performed.

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